# THE PHYSIOLOGICAL ACTION OF CERTAIN PLASMOLYZING AGENTS.

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## INTRODUCTORY.

IN the course of an investigation in which the writer was concerned regarding the physiological action of certain sodium salts, various degrees of destructiveness were observed. In some of the weakest salts, it seemed possible that the osmotic action as well as the chemically harmful properties of the substances might play a rôle, so concentrated were the solutions in which the test plants first survived. It seemed to be very desirable to analyze, if possible, the injurious physiological action of some of the commoner and less harmful salts into its osmotic (physical) and toxic (chemical) components, should it appear that both contribute to the results seen.

The desirability of some clearer understanding of the action of these substances seemed the greater by reason of the frequent use as plasmolyzing agents to which certain of them have long been put by plant physiologists. Sodium chlorid and potassium nitrate, especially, have been much employed in this way. In thus using them, botanists have followed the lead of De Vries, (1) who first applied these substances in this way, and have assumed them to be essentially harmless, as asserted by De Vries (p. 12). He says, "Es ist eine sehr verbreitete irrthümliche Ansicht, dass concentrirte Salzlösungen (z. B., eine Kochsalzlösung von 10–20 %) für des Leben der Pflanzenzelle gefährlich seien." Davenport (2) more recently says, "It is not easy to find a reagent of which we may be certain that it acts only osmotically. NaCl is probably more generally useful in this way than any other substance."

As far as the writer knows, no attempt has been made hitherto to analyze thus the action of a substance, beyond the single

one made by Plateau (3), who used as test objects animals, mainly invertebrates. His experiments turned out unfortunately on account of the fundamental error made in using as comparative solutions percentage concentrations. Recently, Davenport (2) has outlined a method of analysis, in itself correct, but likely to lead to error in many cases through the choice of sodium chlorid as a standard substance assumed to exert a purely osmotic effect.

## METHOD.

In attempting to analyze the injurious action of a compound into its osmotic and its toxic constituents, a standard solution which has a known osmotic action, and is not open to the suspicion of being in any degree toxic, may be selected for purposes of comparison.

As such a standard substance, I have selected cane sugar. It occurs normally in plant cells, often in high concentration, twenty per cent. having been observed in the sugar beet (4). As has been often proved, it is a most excellent plant food when supplied in cultures. In searching through the results of investigators who have made use of this substance in cultures, I have failed to find in any case toxic action attributed to cane sugar. In higher concentrations it proves harmful on exposure of plants to its action for long periods of time, an effect that seems to be amply accounted for by the osmotic properties of such solutions. Moreover, it diffuses rather slowly, and is less liable to injure the organism by the sudden withdrawal of water than is the case with substances of more rapid osmotic action. A limited opportunity for the cell to accommodate itself to the changing concentration is gained in the more slowly diffusing substances.

Having chosen cane sugar as a standard of pure osmotic effect, solutions were made up on the basis of a molecular weight of substance in the requisite numbers of liters required to give the concentrations desired. Tufts of Spirogyra filaments, first rinsed thoroughly in duplicate solutions to prevent dilution of those used in the experiments, were placed in the desired concentra-

tions and left for twenty-four hours. At the end of this time, the condition of the algæ was examined into with greatest care, and the strongest concentration in which they survived was noted. This limit I have termed the boundary concentration, and regard it as a measure of the purely osmotic action capable of being sustained by Spirogyra. Having determined this point, I calculated, by the use of methods in no way involving the living cell, the concentrations of the solutions of other substances to be studied, which have an osmotic value equal to that of the experimental boundary concentration of cane sugar. The next step was to determine experimentally as just described, the boundary concentration of each substance under study.

If the algæ survived in a concentration greater than that calculated from the value of cane sugar, the conclusion would necessarily follow that the substance in question was less harmful to the plants than sugar. This condition of things was in no case realized. If the algæ first survived in the calculated concentration, the action of the substance would be purely osmotic and equal to that of cane sugar. If the algæ should first survive in a concentration more dilute than the calculated boundary concentration, the substance in question would be more harmful than sugar.

In the realization of the third case, injury by one or both of two possible methods might be wrought; first, by a very rapid extraction of water from the cell, violence might be done to the protoplast through the lack of opportunity for the organism to accommodate itself to the change; second, toxic action due to the chemical interference of the substance in solution with the molecules of living substance might also take place. In each special case, it would be necessary to ascertain the kind of injury operating. In doing this certain plain considerations should be borne in mind. Should Spirogyra be found to survive at a concentration greater than that causing plasmolysis and less than the calculated boundary, the deleterious action would in great probability be due to the osmotic properties of the solution, a point again to be touched on in this paper. If, however, the

algæ should die in a concentration less in osmotic value than the cell sap, *i. e.*, at a concentration weaker than the plasmolyzing strength, death could hardly be attributed to the water-extracting properties of the molecules or ions.

## EXPERIMENTAL RESULTS.

I have studied, according to the method just described, those substances which have been most used by biologists as plasmolyzing agents, cane sugar, glycerin, sodium chlorid, and potassium nitrate.

The numerical data obtained are brought together for more ready reference in the following table. Three important points determined for each substance are presented: (1) the concentration of the solution in which plasmolysis begins in the Spirogyra studied stated in gram-molecules of the substance per liter of water; (2) the calculated boundary concentration, assuming a purely osmotic action of a type not more injurious than that seen in cane sugar; (3) the boundary concentration found by experiment.

The temperature varied between 22° and 30° C. as limits. Although the significance of relationships is lost when the concentrations are so stated, I have added percentage values to facilitate comparison with results obtained by other investigators. As an "antidote" for the percentage values, the molecular weights of the substances under study are also added.

| Substance | Mol. wt. | Plasmolyzing concentration | | Calculated boundary concentration | | Experimental boundary concentration | |
|---|---|---|---|---|---|---|---|
| | | gm. mol. per l. | Per cent. | gm. mol. per l. | Per cent. | gm. mol. per l. | Per cent. |
| Cane sugar | 342.0 | 0.33 (1/3) | 11.4 | | | 0.75 (3/4) | 25.7 |
| Glycerin | 92.0 | 0.33 (1/3) | 3.1 | 0.75 (3/4) | 6.9 | 0.50 (1/2) | 4.60 |
| Sodium chlorid | 58.5 | 0.25 (1/4) | 1.5 | 0.46 | 2.7 | 0.10 (1/10) | 0.58 |
| Potassium nitrate | 101.0 | 0.25 (1/4) | 2.5 | 0.47 | 4.5 | 0.06 (1/16) | 0.63 |

As appears in this table, Spirogyra just begins to plasmolyze in a cane sugar solution having a concentration of 1/3 gm. mol

per liter, and the most concentrated solution in which it can survive is ¾ gm. mol. Since cane sugar is assumed to have a purely osmotic action, we have in ¾ gm. mol. of cane sugar per liter of water an osmotic quantity just small enough to permit life, and any other substance acting likewise in a purely osmotic manner will have as its boundary concentration, that concentration which is equal in its osmotic action to a solution of cane sugar containing ¾ gm. mol. per liter.

We are able, assuming purely osmotic action, to calculate the concentration in which the algæ will just survive. The boundary concentration of cane sugar, the osmotic value of cane sugar and that of the substance in question, being given, the boundary concentration would be found to be related to that for cane sugar inversely as the known osmotic values, according to the proportion

$$S : x :: ox : oS$$

in which $S$ is the boundary solution of cane sugar; $x$ the boundary solution of the substance; $ox$ the osmotic value of the substance; and $oS$ the osmotic value of cane sugar.

Osmotic pressure is known to depend directly on the number of molecules or part molecules, ions, in the solution, and whatever be the size of the molecules, an equal number will produce an equal osmotic pressure. Since the glycerin molecule undergoes no splitting into ions, like fractions of a gram molecule of cane sugar and of glycerin per liter of water should have the same osmotic value. Hence, assuming physiological properties like those of cane sugar the theoretical boundary concentrations should be the same, ¾ gm. mol. per liter. It is seen, however, that Spirogyra is not able to survive under the conditions of the experiment, until a solution containing but one-half gm. mol. per liter is reached. It should be noted, however, that this concentration is stronger than that first causing plasmolysis.

Why is glycerin more harmful than cane sugar? In spite of repeated experiments, no change of position could be determined for the boundary concentration. Since the Spirogyra still survives in a concentration stronger osmotically than the cell sap,

a toxic action seems hardly probable. It has been long known to physiologists that glycerin (5) penetrates the cell wall and the protoplast very quickly (6) and can be demonstrated in the cell sap. Thus plasmolysis is rapidly diminished. This speaks for the rapid penetration of the cell by glycerin and the question suggests itself whether the consequent loss of time to accommodate itself to the decided osmotic changes brought about by the solution may not account in some measure for the greater harmfulness seen in glycerin. This question, however, will receive further attention.

In the case of sodium chlorid, one finds a different condition of things. As appears in the table of results, common salt, NaCl, plasmolyzes Spirogyra cells first at a concentration of $\frac{1}{4}$ gm. mol., and first can be survived when containing $\frac{1}{10}$ gm. mol. only. If we assume for sodium chlorid a purely osmotic action, we are able, by making use of the action of cane sugar as a standard of osmotic effect, to calculate the corresponding concentration of sodium chlorid that should just permit the survival of the alga.

Since the molecules split up in large proportion into ions, each of which exerts an osmotic effect equal to that of a molecule not so split, it is necessary to have recourse to the experimental results of physical chemistry. On the basis of the molecular conductivity of salt solutions, it is possible to calculate the percentage of salt molecules dissociated in a solution of a given strength. The electrical conductivity at the given volume ($\mu_v$) divided by the electrical conductivity at an infinite dilution ($\mu_\infty$) gives the degree of dissociation. Since the electrical conductivities are not worked out for all boundary concentrations reached in this study, it has been found necessary to use the closest possible approximation. The error thus occasioned, however, is believed to be not sufficiently large to detract materially from the usefulness of the results, since it is impossible to eliminate many minor inaccuracies when working on a question of this nature.

By using tables of molecular conductivities of NaCl, brought

together perhaps in the most complete and convenient form in the work of Landolt and Börnstein (7), one can readily estimate the number of undissociated molecules and of ions in the solution. This number will express the osmotic value of salt when that of cane sugar is taken as one. Knowing, therefore, the boundary concentration of sugar and the osmotic value of NaCl at the different degrees of dilution, we are able to find a theoretical boundary concentration for NaCl, assuming for it a purely osmotic action.

As appears in the table, Spirogyra first survives in NaCl at a concentration of 0.1 gm. mol. per liter instead of 0.46 gm. mol., the approximate boundary required by purely osmotic action. Since the plants succumb in a solution of NaCl hardly one-fourth as concentrated as would be required by pure osmotic action, as seen in sugar, either the method of exerting this osmotic effect is much more disastrous than is seen in the case of sugar, or some form of toxic activity is here exerted by the NaCl. Since the boundary concentration is so dilute (0.1 gm. mol. per l.), it seems extremely improbable that the mere withdrawal of water from the cell sap by the salt solution could exert a harmful effect commensurate with the numerical disparity here observed.

In the case of potassium nitrate ($KNO_3$), a result very similar to that seen in the case of NaCl was obtained. The salts dissociate in about the same degree under like conditions. A concentration of $KNO_3$ equal osmotically to the boundary concentration of sugar would be about 0.47 gm. mol. per liter. If the physiological action of $KNO_3$ were totally osmotic the Spirogyra should first survive in about this concentration. The boundary concentration of $KNO_3$ determined experimentally was found to be $\frac{1}{16}$ gm. mol. per liter. With an osmotic activity very nearly identical with NaCl, a boundary is found at a greater dilution, indicating a degree of toxic activity somewhat greater than that of NaCl.

In the course of a study by Dr. Kahlenberg and myself of the sodium salts of a number of acids, mainly organic, the boundary concentrations for Spirogyra were obtained. A wide range

of variation was found. In but one instance did a salt give a boundary concentration greater than 0.04 gm. mol. per liter (sodium hippurate, 0.08 gm. mol. per. liter), and in a number of cases the algæ did not survive until a solution containing but 1 gm. mol. in 200 liters was reached, viz., sodium cinnamate and sodium protocatechuate. In no case does it appear probable that osmotic action plays any noticeable rôle in bringing about the death of the plants; in all cases, therefore, practically toxic action only can be invoked as the cause of death. Assuming roughly dissociation in both of these salts to be approximately 15 per cent., the osmotic value of these compounds would be 1.15, that of cane sugar taken as unity. On the assumption of purely osmotic action, the theoretical boundary concentration would lie for each of these salts at about 0.65 gm. mol. per liter. Against these roughly calculated values may be contrasted the experimental boundary concentration, 0.005 gm. mol. per liter. The sodium compound most easily tolerated, sodium hippurate, is seen to act chiefly through its toxic properties, the cinnamate and protocatechuate practically entirely so.

It is interesting in this connection to note that boric acid, the much used antiseptic, shows for Spirogyra less toxicity than any of the above mentioned sodium salts.

| Substances | Concentrations in fractions of a gram-molecule per liter. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\infty$ dil. | .06 | 0.1 | 0.17 | 0.25 | 0.33 | 0.46 | 0.50 | 0.67 | 0.75 |
| Cane sugar | | | | | | *p* | | | | *e* |
| Glycerine | | | | | | *p* | | *e* | | *t* |
| NaCl | | | *e* | | *p* | | *t* | | | |
| $KNO_3$ | | *e* | | | *p* | | *t* | | | |
| Sodium hippurate | | | *e* | | | | | | *t* | |
| Sodium cinnamate | *e* | | | | | | | | *t* | |

In closing this review of the experimental details, a diagram is given which presents in a somewhat more graphic form the

cardinal numerical relations which have served as a basis for the discussion. In this diagram, *p* stands beneath the concentration just beginning to call forth plasmolysis; *t* beneath the concentration representing the theoretical boundary concentration; *e* beneath the concentration experimentally determined to be the boundary concentration.

In view of the results above presented, it appears that plants may be fatally affected both by solutions acting osmotically and by solutions acting through their chemical properties.

In the case of solutions acting osmotically and by this method causing death, it should be pointed out that while osmosis furnishes the means, the operation of which leads to a fatal outcome, chemical processes may very well be here involved. One may imagine that the attraction of the sugar molecules for water results in the removal of water more or less rapidly from the organism enclosed within the cell wall. At first, as the cell sap is relatively dilute, it parts with its water to the larger mass of plasmolytic solution without at a relatively rapid rate. The substances dissolved in the cell sap not being yielded up, the cell sap concentration rises. If this process does not continue beyond a certain point, the organism is able, when osmotic equilibrium is reached, to retain enough water to preserve its organization and to sustain for weeks, or even months, a quiescent existence. If, however, an equilibrium between the cell sap and the surrounding medium is not reached until the water necessary to the maintenance of the integrity of the substance and of the processes of life is encroached upon, death must result and probably in the last analysis through chemical changes. Hence, one may imagine that cane sugar in a solution of too great concentration in time will kill the cell through its interference with the chemical integrity of the substance or processes of the cell exerted along the lines of osmotic activity.

In the case of the other compounds working through their toxic properties, the penetration of the substance into the cell is probably followed more or less promptly by chemical changes due to interference with the compounds essential to life, and

death results without any marked withdrawal of water from the cell substance.

In conclusion, it may now be safely asserted that, whereas there exist very many plants which suffer when removed suddenly from salt water to fresh, and appear to be dependent for life itself on the presence of this substance in the substratum, it seems certain that for Spirogyra, sodium chlorid and potassium nitrate act as poisons. It is my opinion, based on a considerable number of experiments not detailed here, that all sodium and potassium salts injure Spirogyra through their chemical properties chiefly, and exert but a minimal harmful influence through their osmotic properties.

In this discussion, I have assumed the accuracy of the classic conclusion that the plasmolytic method as used by De Vries, Pfeffer, and others gives an essentially correct measure of the concentration of the cell sap.

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